

OPTICAL DISCS FOR MEASURING ANALYTES

This application claims priority from U.S. Provisional Application Serial No. 60/254,394, filed December 8, 2000; U.S. Provisional Application Serial No. 60/255,233, filed December 12, 2000; U.S. Provisional Application Serial No. 60/293,917, filed May 24, 2001; U.S. Provisional Application Serial No. 60/294,051, filed May 29, 2001; U.S. Provisional Application Serial No. 60/294,052, filed May 29, 2001; U.S. Provisional Application Serial No. 60/303,437, filed July 6, 2001; U.S. Provisional Application Serial No. 60/306,226, filed July 18, 2001; and U.S. Provisional Application Serial No. 60/323,405, filed September 19, 2001.

FIELD OF THE INVENTION

This invention relates to optical discs configured to receive analytes which can be detected by a standard optical disc reader or an optical disc reader modified therefrom.

BACKGROUND OF THE INVENTION

Optical discs have been used for detection and characterization of biological and chemical samples. For instance, see WO 96/09548 (Gordon), EP A 392475 (Idemitsu), EP A 417 305 (Idemitsu), EP A 504432 (Idemitsu), and WO 98/12559 (Demers), all of which are incorporated herein by reference. Other examples of using optical discs to detect investigational samples can be found in U.S. Provisional Application 60/252,725, entitled "Optical Bio-Disc Including Microfluidic Circuit For Separation And Quantification Of Agglutinated Microparticles Or Cells And Methods Relating Thereto," U.S. Provisional Application No. 60/252,726, entitled "Bioactive Solid Phase For Specific Cell Capture And Optical Bio-Disc Including Same," and U.S. Provisional Application No. 60/257,705, entitled "Surface Assembly For Immobilizing DNA Capture Probes And Bead-Based Assay Including Optical Bio-Discs And Methods Relating Thereto," all of which are incorporated herein by reference.

These previously described optical discs, however, are not designed to be read by standard optical disc readers, such as standard CD or DVD readers. For instance, the optical discs disclosed in EP A 392475 (Idemitsu), EP A 417 305 (Idemitsu) and EP A 504432 (Idemitsu) require the use of two optical detectors, one to detect the tracking information and

the other to detect surface structures. In contrast, reading a standard CD or DVD needs only one optical detector.

Therefore, there is a need to design and manufacture an optical disc configured to receive an investigational sample which can be detected by a standard optical disc reader or an optical disc reader modified therefrom.

Over the past decade, scanning laser microscopy (SLM) has revolutionized life science imaging. However, SLM demands expensive and specialized optical equipment. Consequently, there exists a need to provide an inexpensive, generic device which can carry out laser scanning over microscopic specimens.

The present invention discovers that the minimum mechanical requirements for SLM, i.e. laser, focusing and detection optics, precision scanning means, and computer interface, may all be provided by a standard optical disc reader or an optical disc reader modified therefrom. Therefore, it is desirable to create an optical disc which can hold a microscopic sample that can be scanned by a standard optical disc reader. Such an optical disc presents a marked advantage over existing SLM technologies.

In order for a standard optical disc reader to operate an optical disc, the optical disc reader is typically required to be able to (1) accurately focus above the operational surface of the optical disc, (2) accurately follow the spiral track or utilize some form of uniform radial movement across the optical disc surface, (3) recover enough information to facilitate a form of speed control, such as CAV, CLV, VBR, CBR, or ZCLV, (4) maintain proper power control by logical information gathered from the optical disc or by signal patterns detected in the operational surface of the optical disc, and (5) respond to logic information that is used to control, for example, the position of the objective assembly, the speed of rotation or the focusing position of the laser beam.

A typical optical disc system uses elements of the optical medium itself to satisfy at least some of these operational requirements. For instance, in a typical CD, the disc substrate is impressed with a spiral track made up of a series of embossed or impressed pits and lands. Light reflected from these pits and lands can be used to generate signals. These signals are used by the optical disc reader to maintain proper focusing and tracking. In a CD-R disc, a wobble groove is used to generate operational signals during disc recording. Dye marks are created during disc recording, and these dye marks may provide the requisite tracking structures during subsequent reading. Generally, under each of conventional optical disc

standards, the structures that encode data may simultaneously serve to provide operational signals that enable an optical disc reader to operate the optical disc.

Conventional optical disc standards make no provision with respect to acquisition of information from investigational features, such as biological or chemical specimens, that are disposed on the disc. Investigational features disposed on the disc may disrupt the tracking of the disc. In addition, investigational features may be sufficiently separated from operational structures, therefore preventing an optical disc reader from tracking the disc and detecting the investigational features concurrently and discriminably.

Therefore, there is a need to provide an optical disc which allows an optical disc reader to detect the investigational features without disrupting the tracking of the disc. There also exists a need to provide an optical disc which allows an optical disc reader to track the disc and read the investigational features concurrently and discriminably.

SUMMARY OF THE INVENTION

In one embodiment, an optical disc assembly is configured to associate with an analyte which can be detected by a standard optical disc reader or an optical disc reader modified therefrom.

In another embodiment, an optical disc assembly is configured to associate with an analyte, and the association with the analyte does not prevent an optical disc reader from tracking the disc assembly.

In yet another embodiment, an optical disc assembly, which is associated with an analyte, is configured to enable an optical disc reader to track the disc assembly and detect the analyte concurrently and discriminably.

In accordance with one aspect of this invention, the optical disc assembly includes (1) optically readable structures which are trackable by an optical disc reader and which have encoded speed information enabling the optical disc reader to rotate the optical disc at a speed that is determinable from the speed information; and (2) an analyte section capable of receiving an analyte which can be detected by the optical disc reader. The optically readable structures may be impressed in the operational surface and coated with a reflective layer. The optical disc reader may be a CD or DVD reader. The optically readable structures can be in a CD format or a DVD format.

The analyte section may include at least one analyte chamber located within the disc assembly, such as between the operational layer and another layer of the disc assembly. At

least one channel may be created in one of the layers of the disc assembly to allow the analyte or other components to enter or exit the analyte chamber. The channel may include a valve regulated by optically readable data encoded in the optical disc assembly. In one embodiment, the analyte section includes a plurality of analyte chambers, each chamber
5 being connected to at least a channel.

In one embodiment, design information of the optical disc assembly is encoded in the disc assembly.

In another embodiment, the disc assembly is a reverse disc including a lens layer capable of focusing the laser beam on the operational surface which is located at the laser-
10 proximal surface of the operational layer.

In yet another embodiment, the operational surface in the reverse disc includes a cut-away area which either lacks operational structures or includes modified operational structures. In addition, the cut-away area may lack reflective coatings.

In a preferred embodiment, the analyte chamber includes a surface which is located
15 within 15 micrometers from the operational surface, preferably located within 15 micrometers from the cut-away area, and which preferably is capable of receiving the analyte.

In another preferred embodiment, the disc assembly is a forward disc. The operational surface is at the laser-distal surface of the operational layer and coated with a semi-transmissive, semi-reflective layer. There is a second layer which is laser-distal to the operational layer and which is coated with another reflective layer. Preferably, the
20 operational surface includes a cut-away area, and the analyte chamber includes a surface which is located within 15 micrometers from the cut-away area. More preferably, the analyte chamber includes the cut-away area. The second layer may lack optically readable structures
25 that have encoded tracking information.

In one embodiment, the analyte section contains the analyte which may be a biological or chemical sample. Preferably, the analyte is located within 15 micrometers from the operational surface.

In yet another embodiment, the analyte section is embedded in the disc assembly and
30 includes a surface which is located within 15 micrometers from the operational surface and which preferably is capable of receiving the analyte.

In one embodiment, the disc assembly has encoded information for conducting an assay on the analyte.

In accordance with yet another aspect of this invention, the optical disc assembly includes (1) a hologram which contains optically readable features that have encoded (a) tracking information, and (b) speed information enabling an optical disc reader to rotate the optical disc at a speed that is determinable from the speed information; and (2) an analyte section capable of receiving an analyte which can be detected by the optical disc reader. The
5 hologram may be reflective or replaceable. The optical disc reader may be a CD or DVD reader.

In one embodiment, at least part of the image plane of the hologram is located within the analyte section. The analyte section may include the analyte which may be positioned
10 within 15 micrometers from the image plane of the hologram. The laser beam may be focused on the image plane of the hologram.

In accordance with another aspect of this invention, an analyte held in the analyte section of the optical disc assembly is detected using a CD or DVD reader. The detection includes the steps of (1) introducing the optical disc assembly into the CD or DVD reader; (2)
15 reading the optically disc assembly; and (3) obtaining a signal which is indicative of the presence of the analyte. Preferably, the signal thus obtained is a quad sum signal.

BRIEF DESCRIPTION OF THE DRAWINGS

All the drawings used herein are given by way of illustration, not limitation, and all
20 the drawing are not drawn to scale.

FIG. 1 demonstrates the functional components of an optical disc assembly according to one embodiment of the present invention.

FIG. 2 shows a cross-sectional view of an optical stack including three layers of refractive material.

FIG. 3 is a cross-sectional view of a compact disc recordable (CD-R) including a polycarbonate layer, a dye layer, and a reflective layer.

FIG. 4 is a diagram showing the geometry of a wobbled groove as it is embossed in an operational surface.

FIG. 5 shows a common arrangement of a reading laser beam relative to a spinning
30 optical disc.

FIG. 6 shows wobbled grooves in a forward wobble optical disc, wherein the wobble grooves are embossed in the laser-distal surface of the operational layer.

FIG. 7 shows a forward wobble disc assembly containing wobbled grooves coated with a reflective layer, wherein the operational surface has a cut-away area.

FIG. 8 shows a cross-sectional view of a forward wobble optical disc assembly with an investigational feature placed on a cut-away area.

5 FIG. 9 demonstrates a reverse wobble optical disc assembly, wherein the wobble grooves are embossed in the laser-proximal surface of the operational layer.

FIG. 10 presents a cross-sectional view of a reverse wobble disc assembly with an investigational feature placed in the analyte section.

10 FIG. 11 compares the mastering process for making a forward disc with the mastering process for making a reverse disc.

FIG. 12 further compares the mastering process for making a forward disc to the mastering process for making a reverse disc.

FIG. 13 illustrates a reverse optical disc assembly.

FIG. 14 illustrates an optical pickup used in one embodiment of the present invention.

15 FIG. 15 depicts a quad detector used in one embodiment of the present invention.

FIG. 16 depicts the positions of investigational structures with respect to the tracks of operational structures.

FIG. 17 shows the HF signals acquired from the tracks illustrated in FIG.16.

20 FIG. 18 shows an optical disc assembly including a gnat wing in an inspection channel.

FIG. 19 depicts the position of the gnat wing with respect to the tracks of the operational structures.

FIG. 20 shows the HF signals acquired from a track of the operational structures illustrated in FIG. 19.

25 FIG. 21 depicts the position of the gnat wing with respect to the tracks of the operational structures.

FIG. 22 demonstrates the HF signals acquired from a series of consecutive tracks of the operational structures illustrated in FIG. 21.

30 FIG. 23 depicts the position of the gnat wing with respect to the tracks of the operational structures.

FIG. 24 shows a high density compilation of the HF signals acquired from the tracks across the gnat wing.

FIG. 25 shows an objective lens focusing mechanism as used in one embodiment of the present invention.

FIG. 26 illustrates an optical disc assembly containing a reflective hologram.

FIG. 27 illustrates the data and surface organization of a zoned constant linear
5 velocity (ZCLV) format.

FIG. 28 shows an enlarged perspective view of a portion of a section of a ZCLV disc, wherein the portion has a pre-groove area followed by a wobble groove area.

FIG. 29 shows investigational features placed in a section in a ZCLV disc.

FIG. 30 is an example of a forward disc, wherein the operational structures are coated
10 with a semi-reflective layer, and investigational features are held laser-distal to the semi-reflective layer.

FIG. 31 shows a example of a forward disc which permits the laser light to pass through the disc to a top detector.

FIG. 32 depicts a reverse optical disc assembly which includes fluidic channels placed
15 either above or below the operational surface.

FIG. 33 shows another reverse optical disc assembly in which the operational surface has a cut-away area and fluidic channels are placed either above or below the cut-away area.

FIG. 34 illustrates an example of a reverse optical disc assembly which includes channels and an analyte chamber.

FIG. 35 depicts a forward optical disc assembly which includes fluidic channels
20 placed either above or below the operational surface.

FIG. 36 shows another forward optical disc assembly in which the operational surface has a cut-away area and fluidic channels are placed either above or below the cut-away area.

FIG. 37 shows a forward optical disc assembly, wherein channels are contained in a
25 lens layer.

FIG. 38 shows a forward disc with channels placed above or below a cut-away area.

FIG. 39 shows a forward disc assembly having a cut-away area.

FIG. 40 illustrates an exploded perspective of the principle layers in a forward disc assembly according to one embodiment of the present invention.

FIG. 41 shows a design of an operational layer.
30

FIG. 42 shows a design of an adhesive layer.

DESCRIPTION OF EMBODIMENTS OF THE INVENTION

This invention relates to an optical disc assembly configured to receive an analyte of interest which can be detected by an optical disc reader. The analyte of interest may be a physical specimen, such as a biological, chemical, or biochemical specimen, or a product
5 produced by a biological or chemical reaction carried out in the optical disc assembly. The optical disc reader may be a CD reader or a DVD reader. The optical disc reader may be a standard optical disc reader or an optical disc reader modified therefrom. The optical disc assembly may be designed so that the association of the analyte with the disc assembly does not prevent the optical disc reader from tracking the disc assembly or performing other
10 operational functions.

As used herein, various surfaces in an optical disc assembly can be numbered or named according to the order in which the light beam of the optical disc reader strikes or passes through them upon first occurrence. For example, when a compact disc (CD) is read by a CD reader, the laser beam of the CD reader first enters the disc through a surface of a polycarbonate layer. The polycarbonate layer also is known as the polycarbonate disc or the
15 substrate. This surface is referred to as the first surface or the laser-proximal surface of the polycarbonate layer. The laser beam proceeds through the polycarbonate layer and comes out through another surface of the polycarbonate layer. This latter surface is referred to as the second or the laser-distal surface of the polycarbonate layer. The laser beam proceeds to a reflective layer which is usually made of gold or other reflective material. The reflective
20 layer is laser-distal to the polycarbonate layer. The laser beam is reflected back from the reflective layer, passing through the polycarbonate layer and entering a detector in the CD reader.

When a CD-R disc is read by a CD-R reader, the laser beam of the reader enters the
25 CD-R disc through the first surface of a polycarbonate layer. The laser beam proceeds through the polycarbonate layer and enters into a dye layer which is laid on the tracks embossed or impressed in the laser-distal surface of the polycarbonate layer. The polycarbonate layer is laser-proximal to the dye layer. The back surface of a CD-R disc, upon which labels or markings are laid, is referred to as the back plane. The back plane is the
30 most laser-distal surface in the CD-R disc.

As used herein, "operational structures" or "operational features" in an optical disc assembly refer to optically readable structures which are impressed or encoded in the optical disc assembly and which enable an optical disc reader to track, synchronize, or perform other

customized operational functions. Operational structures may act as phase components or provide interference patterns. Operational structures may have encoded speed information that enables the optical disc reader to rotate the disc assembly at a speed determinable from the speed information. Light returned from or passed over operational structures can be
5 acquired by the optical disc reader to generate operational signals. These operational signals are used by the optical disc reader to track, focus, synchronize, or perform other operational functions.

Operational structures may be imprinted or impressed in a surface of a layer in the disc assembly. Such a layer is referred to as an "operational layer," and such a surface is
10 referred to as an "operational surface." In a typical CD disc, the operational layer is the polycarbonate disc, and the operational surface is the laser-distal surface of the polycarbonate disc. An optical disc assembly may have more than one operational layer or operational surface. Preferred operational structures include, but are not limited to, wobble grooves, pits and lands, dye marks, or any combination thereof.

Operational structures may be encoded in a hologram. Light returned from or
15 transmitted through the hologram can create an image plane within which the encoded operational structures appear to be positioned. The encoded operational structures, as appeared in the image plane of the hologram, preferably are in the form of wobble grooves, tracks of pits and lands, or any other type of operational structures that may be physically
20 impressed in an optical disc's operational layer.

Operational structures, impressed or encoded, may be in a variety of formats. Suitable formats for this invention include, but are not limited to, CD formats, DVD formats, any combination thereof, or other optical disc formats. CD formats include, but are not limited to, CD-ROM, CD-R and CD-RW formats. DVD formats include, but are not limited
25 to, DVD-R, DVD-RW and DVD-RAM formats. As appreciated by one of skill in the art, other CD or DVD formats or other optical disc formats, including those that have been or will be developed in the future, may be used in the present invention.

"Investigational structures" or "investigational features" refers to the structures, features or items that are placed in an optical disc assembly to be examined. An
30 investigational structure or feature may be an analyte which includes a physical specimen, such as a biological or chemical sample, or a product produced by a biological or chemical reaction conducted in the optical disc assembly. An investigational structure or feature may also be part of an analyte. Investigational structures or features usually cannot provide

operational information. Investigational structures or features typically are not imprinted or impressed in the optical disc assembly. They usually are not encoded in a hologram.

Preferably, investigational structures or features are replaceably disposed in the optical disc assembly. Investigational structures or features may be chemical, bio-chemical, or biological in nature. They may also be signal or reporter elements such as beads.

Association of investigational structures with an optical disc assembly of the present invention does not prevent the optical disc reader from operating the optical disc assembly. In order to operate an optical disc assembly, the optical disc reader usually needs to (1) accurately focus above the operational surface of the disc assembly, (2) accurately track the operational surface or use some form of radial movement across the disc surface, (3) maintain a form of speed control, (4) maintain proper power control by logical information gathered from the disc assembly, and (5) respond to logic information that may be used to control, for example, the position of the objective assembly, the speed of rotation, or the focusing position of the laser beam.

An analyte that is disposed in the optical disc assembly of the present invention can be read or detected by an optical disc reader. As used herein, an analyte can be read or detected by an optical disc reader if the optical disc reader can generate at least a signal indicative of the presence of the analyte. The present invention also contemplates the use of the optical disc reader to generate signals indicative of other properties of the analyte, such as the concentration or dimension of the analyte.

In accordance with one aspect of the present invention, the optical disc assembly includes at least an operational layer and at least an analyte section. The operational layer contains an operational surface in which operational structures are impressed. The operational structures have encoded operational information which enables the optical disc reader to operate the disc assembly. In particular, the operational structures may have encoded tracking information that allows the disc reader to track the operational structures. The operational structures may also have encoded speed information enabling the disc reader to rotate the disc assembly at a speed determinable from the encoded speed information. Preferably, the operational structures are coated with a reflective layer containing a reflective material, such as metal, aluminum, gold, silver, or silicon. As used herein, a reflective layer can be semi-reflective and semi-transmissive. Light reflected from the reflective layer can be acquired by the disc reader to generate operational signals for focusing, tracking, or

performing other operational functions. The operational layer may be a hologram in which the operational structures are encoded.

Speed information encoded in the operational structures allows an optical disc reader to rotate the optical disc assembly at a determinable speed. Speed information may be encoded in frame synchronization words which allow the optical disc reader to adjust the disc speed to keep a desired data rate. The speed information may also be encoded in a wobble groove. The wobble groove can produce signals useful for regulating the disc speed. In addition, special marks or logic information can be used to provide speed information. In one embodiment, the optical disc reader rotates the optical disc assembly with a constant linear velocity.

The analyte section is configured to receive at least an analyte of interest. The analyte section may be embedded in the disc assembly. It may be positioned between two layers of the disc assembly. It may also be located within a layer in the optical disc assembly. As used herein, a layer in a disc assembly refers to a thickness of material. For instance, a layer may be a substrate disc or a coating of reflective material. A layer may also be an insert which can be assembled into another layer in the disc assembly. A layer may be flat or not flat. A layer may be homogeneous or non-homogenous. The depth of a layer may be uniform or not uniform. A layer may be an assembly of several parts. The analyte section may be the most laser-distal or the most laser proximal structure in the disc assembly. The analyte section may include channels, microfluidic channels, chambers, cavities, or other structures that are designed for the manipulation, creation, or retention of the analyte of interest or the investigational structure. The analyte section may also be referred to as the component layer. An optical disc assembly may have more than one analyte section. The analyte section and the operational layer can be intermixed so long as the optical disc reader can retain control of focusing, tracking, velocity and other operational functions. Analytes or investigational structures can be in the nanometer, micrometer or millimeter range, and can be disposed, modified or created in the analyte section.

Preferably, the optical disc assembly includes a lens layer. The lens layer may be used to focus the laser beam, for instance, on either the operational structures, or the investigational structures. In one embodiment, the operational layer may function as a lens layer. In such a case, the operational structures are embossed in the laser-distal surface of the operational layer. The refractive index of the lens layer can be selected to perform the desired focusing function. The lens layer typically is laser-proximal to the operational or

investigational structures. Preferably, the lens layer includes a material selected from the group consisting of plastic and glass. More preferably, the lens layer consists of plastic, such as polycarbonate or polystyrene. In a highly preferred embodiment, the lens layer consists of polycarbonate. Other examples of material suitable for constructing a lens layer include

5 polymethylacrylic, polyethylene, polypropylene, polyacrylate, polymethyl-methacrylate, polyvinylchloride, polytetrafluoroethylene, polyacetal, polysulfone, celluloseacetate, cellulosenitrate, nitrocellulose, or any mixtures or combinations thereof.

The optical disc assembly of the present invention preferably approximates the dimensions of a unitary disc. For instance, the disc assembly may have a radial diameter

10 between about 110 mm and 130 mm, including the range from 75 mm to 85 mm, and preferably 120 mm. Preferably, the disc assembly has a depth or thickness between about 0.8 mm and 2.4 mm, such as between 1.0 mm and 1.4 mm. More preferably, the disc assembly has a depth or thickness between 1.1 mm and 1.3 mm, including 1.2 mm. In one

15 embodiment, the disc assembly may have a thickness of about 0.6 mm. The disc assembly may be flat or not flat, circular or non-circular. The disc assembly may have a central hole through which the disc assembly can be coupled to a disc reader.

With reference to FIG. 1, there is shown a diagram illustrating one embodiment of the present invention. The optical disc assembly has at least an operational layer 338, a component layer 336, and a lens layer 340. The disc assembly encompasses the focusing range of the laser used by the disc reader. The operational layer 338 contains the operational structures that are used by the disc reader to track the disc assembly. The operational structures may create an interference pattern or other patterns that provide operational functionality to the disc reader. The component layer 336 contains the investigational structures as well as other structures that are related to the manipulation, creation, or retention

20 of the investigational structures. The component layer may include microfluidic channels. The lens layer 340 may focus the laser beam either on the operational structures or on the investigational structures. The component layer and the operational layer may be intermixed or overlapped so long as the disc reader can retain control of the focusing, tracking and speed. The measurement area 342 represents the area which are readable by the disc reader's laser,

25 and the laser's focal point can roam across the measurement area. The measurement area may encompass any of the component layer, the operational layer, the lens layer, or a portion thereof.

30

Different layers in an optical disc assembly may have different optical properties and can impose different optical effects on the light beam that passes through these layers. These optical effects include, for example, reflection, refraction, transmission, or absorption. FIG. 2 is a simplified example showing the effects of different layers on the passage of a light beam.

5 The optical disc 102 in FIG. 2 includes three layers of refractive material, 104, 106 and 108. The refractive properties of these layers may differ based on their compositions. The different refractive properties create changes in the light beam 110 as the light beam passes through these layers. The layers may be named in order of their first contact with the light beam 110. The light beam 110 enters through the bottom surface 112 and exits at the
10 top surface 118. Therefore, layer 104 may be referred to as the most laser-proximal layer, and layer 108 as the most laser-distal layer. Layer 106 is laser-distal to layer 104, and laser-proximal to layer 108.

The light beam 110 enters layer 104 at surface 112. The light beam is bent and slowed because of the refractive property of layer 104. The light beam exits layer 104 at
15 surface 114, and then enters layer 106 which has a different refractive property, therefore further altering the angle and speed of the light beam. As the light beam exits layer 106, it enters layer 108 at surface 116 with a further changed angle and speed. The light beam exits layer 108 at surface 118. Surface 112 is laser-proximal to surface 114 which is laser-proximal to surface 116. Surface 118 is the most laser-distal surface in the optical stack 102.

20 In one embodiment, the optical disc assembly is designed based on a modification of an industry standard design, such as standard CD (including CD-R and CD-RW) or a standard DVD (including DVD-RAM, DVD-R and DVD-RW). The following description is based on modifications of a standard CD-R disc. Similar modifications can be applied to other optical discs, such as CD-RW or DVD discs, as appreciated by one of ordinary skill in
25 the art.

A normal CD-R disc contains a wobble groove utilized by the CD-R drive to spin and track the disc. A similar principal applies to a DVD-type disc. A standard CD-R disc has a polycarbonate operational layer having a depth of about 1.2 mm. The polycarbonate operational layer has at least a wobble groove embossed in the laser-distal surface of the
30 layer. The depth of the wobble groove in a standard CD-R is approximately from 165 nanometers to 230 nanometers. A typical wobble groove is filled with dye. Above the dye layer is a reflective layer which contains gold or other reflective material such as silver. The reflective layer can reflect the laser beam of the disc drive back to a detector in the disc drive.

The laser beam can be focused on the reflective layer. The laser's focus may be adjusted by a focusing servo. The wobble groove functions like a diffraction grating, and is capable of creating a pattern in the reflected laser light. The pattern in the reflected laser light is used by the disc drive to spin and track the disc. In a standard DVD disc, the wobble groove depth
5 can be 50 nanometers.

FIG. 3 exemplifies a CD-R disc with an industry standard design. The disc has an operational layer 120 which is usually made of polycarbonate, a dye layer 122 which contains CD recordable light sensitive dye, and a reflective layer 124 which contains gold, silver or other material that is suitable for reflecting the laser beam 128 back to the detector
10 of the disc drive. The operational layer 120 is capable of focusing the laser beam 128 on the reflective layer 124. As used herein, a layer in an optical disc is capable of focusing a reading beam of an optical disc reader on an object if the reading beam, which is directed to the object, can pass through the layer and become focused on the object while the optical disc reader is operating the optical disc. Preferably, the refractive index of the layer is
15 significantly greater than the refractive index of air, as appreciated by one of skill in the art. For instance, the refractive index of the layer may be 1.55. Other layer or layers may also exist in the optical path of the reading beam to help the reading beam become focused on the object. The wobble groove 126 is shown in FIG. 3.

FIG. 4 demonstrates the geometry of a wobbled groove in a CD-R. As with other figures used herein, FIG. 4 is not drawn to scale. The wobble groove is usually embossed into the laser-distal surface of the operational layer in a CD-R. FIG. 4 shows two wobbled grooves. Measurement A, 132, represents the distance between two adjacent wobble grooves. Measurement B, 134, denotes the width of the top of the wobble groove. Measurement C, 136, represents the width of the bottom of the wobble groove. Measurement
20 D, 138, shows the depth of the groove. The angle E, 140, between the side-wall 142 and the bottom of the wobble groove has an important effect on light that encounters the wobbled groove. The geometry of the wobble groove in a CD-R disc has a significant effect on the performance of the CD-R disc in a high speed writer.

An optical disc spins in a disc reader clockwise when looking down on the disc.
30 From the viewpoint of the reader's laser (looking up), however, the disc is spinning counter-clockwise. FIG. 5 demonstrates a common arrangement of an optical disc in a disc drive. The same arrangement can be employed in the present invention. The laser beam 146 is directed to the optical disc 144 from below. The optical disc 144 spins in a

counter-clockwise direction **148** as seen from below the disc. From above the disc, however, the disc **144** spins in a clockwise direction.

The optical disc of the present invention may be designed based on modifications of a standard CD-R disc. The standard CD-R disc is a "forward" disc, in which the operational structure is positioned at the laser-distal surface of the operational layer. Whereas the
5 operational structure of a forward disc is a wobble groove, the disc is also referred to as a "forward wobble" disc. A forward wobble disc has its wobble groove positioned on the second surface or the laser distal surface of the operational layer.

FIG. 6 illustrates a forward disc **150**. Laser beam **152** enters the operational layer **158**
10 at its first or laser-proximal surface **154**. The light is bent according to the refractive property of layer **158**. The focus of the laser beam **152** encompasses the bottom of wobble groove **160**. Wobble groove **160** is embossed in the second or laser-distal surface **156** of layer **158**. Reference numeral **159** denotes a land of the wobble groove.

FIGs. 7 and 8 illustrate the forward discs that may be employed in the present
15 invention. The forward optical disc assembly **224** in FIG. 7 includes an operational layer **226** which has wobble grooves in its laser-distal surface. The wobble grooves are coated with a reflective layer **228** containing reflective material, such as gold. The reflective layer **228** may be semi-reflective. The operational surface has a cutaway area at location **229** which allows laser light **230** to pass through the wobble grooves and reach another reflective layer **232**.
20 The reflective layer **232**, which contains reflective material, can be deposited on a top cover layer **234** which can provide a stable surface for the reflective layer **232**. Assembly **224** also has an analyte section **236** which is configured to receive analytes of interest. The tracking of the disc is performed by the laser beam **230** when it encounters the reflective layer **228** that covers the wobble grooves. The size and configuration of the cut-away portion **229** is
25 calculated to allow the laser to resume tracking before control of the disc is lost by the disc drive's tracking mechanism. The reflective layer **232** permits the laser beam to return to a detector in the disc reader after the laser beam passes through the cut-away portion **229** to the analyte section **236**.

As used herein, a "cut-away" area refers to an area in the operational surface, wherein
30 the area either lacks operational structures or the operational structures in the area are changed in certain ways. The operational structures in a cut-away area may be deprived of reflective coatings, or be coated with a reflective material having a different reflectivity than the reflective material coated on other regions in the operational surface. In a preferred

embodiment, one surface of the analyte section is adjacent to a cut-away area, or includes a cut-away area.

FIG. 8 shows another forward optical disc which includes an investigational structure 354. The operational layer 344 contains the operational structures which are embossed at its laser-distal surface and coated with a reflective layer 350. The operational layer serves as a lens layer and can focus the laser beam 352 on the operational structures. The analyte section 346 is configured to hold an investigational structure 354, which is within the focal zone 356 of the optical disc reader's laser. The laser beam 352 may pass through a cut-away area 348 and be focused on the investigational structure 354. The cut-away area lacks the reflective coating. The disc assembly has a second reflective layer 360 which is laser-distal to the operational structures and capable of reflecting laser light. The cover 358 is laser-distal to the second reflective layer. The analyte section 346 contains the cut-away area 348 upon which the investigational structure 354 is positioned.

As used herein, a laser's focal zone refers to the range of distance within which the laser's focal point may be positioned. In a standard CD drive, the laser's focal zone is about 25 to 26 micrometers. Thus, the laser's focal point can move above and below a trackable surface in a range of about 12.5 to 13 micrometers. Some variations (about ± 2 micrometers) are allowed for the movement. Accordingly, in a preferred embodiment, the analyte section is capable of positioning an analyte at least within about 15 micrometers from the trackable surface in order for the laser reading beam of the optical disc reader to be focused on the analyte. When a hologram is used to encode operational structures, the analyte preferably is located within about 15 micrometers from the image plane of the hologram. In one embodiment, a surface of the analyte section is located within about 15 micrometers from the trackable surface or the image plane of the hologram. The surface of the analyte section may also be capable of receiving the analyte. The surface of the analyte section may be part of a larger surface of the analyte section. The 15 micrometers limitation may be modified in a modified optical disc reader.

In a preferred embodiment, the optical disc assembly employs a spiral wobble groove to provide operational signals. As used herein, a spiral wobble groove can be considered as a series of wobble grooves connected consecutively to form a spiral track. The depth of the wobble groove is preferably from 50 to 100 nanometers, including 65 nanometers, 70 nanometers, 73 nanometers and 100 nanometers. More preferably, the depth of the wobble groove is approximately $1/8$ of the effective wavelength of the laser light in the layer which is

immediately laser-proximal to the operational surface. Such a depth may provide a strong tracking signal. The groove may also have a depth approximately equal to any odd multiple of 1/8th of the wavelength, such as 3/8ths or 5/8ths. The depth of the groove may remain substantially constant along the wobble groove. The periodic perturbation of the wobble provides the information for tracking. In one embodiment, the spiral groove has a track pitch of approximately 1.6 microns.

Preferably, no dye is laid down in the wobble groove, or the dye is laid down in discrete patterns so as to facilitate drive control during the examination of investigational features. The present invention discovers that a conventional CD-R wobble groove without dye is readable by a standard optical disc reader. The groove can be coated with a layer of reflective material, such as gold, or semi-reflective material. The reflective layer preferably is positioned sufficiently close to the groove structure so as to provide adequate reflection when the laser light focuses on the groove structure. In addition, the pitch and angle of the groove walls can be selected to facilitate the detection of investigational features which are placed on or between various surfaces or layers in the disc.

In accordance with one aspect of the present invention, the optical disc assembly is a "reverse" disc. The operational structures in a reverse disc are imprinted or impressed in the laser-proximal surface of the operational layer. When the operational structure is a wobble groove, the reverse disc may also be referred to as a "reverse wobble" disc. A reverse wobble disc has the wobble groove impressed in the laser proximal surface of the operational layer. A standard CD-R disc has the wobble groove impressed in the laser-distal surface of the operational layer.

FIG. 9 depicts a reverse wobble disc assembly 162. The reverse wobble disc assembly 162 includes an operational layer 164 and a cover 166. The operational layer 164 is further shown in an enlarged view 168. Cover 170 represents the enlarged view of cover 166. Cover 170 provides the most laser-proximal layer in the disc assembly. The laser beam 172 enters cover 170 at its laser-proximal surface 174. The laser beam at surface 174 is bent due to the refractive property of cover 170. The laser exits the cover and is focused on the wobble groove 176 which is embossed in the laser-proximal surface 178 of the operational layer 168. The laser may or may not pass through the wobble groove to reach other layers.

FIG. 10 shows a reverse wobble disc containing an investigational structure. The cover 362 acts as a lens to focus the laser beam 364 onto the operational structures. The laser beam 364 may also be focused on the investigational structure 366, depending the reflectivity

of the investigational structure 366. The investigational structure 366 is held in the analyte section 372 which is between the operational layer 370 and the cover layer 362. The analyte section 372 lies within the laser's focal zone 368 which also encompasses the operational surface. The operational structures are covered by a reflective layer 374.

5 The process for manufacturing a reverse wobble disc is different from the process for making an industry standard forward wobble disc. FIGs. 11 and 12 demonstrate the difference between making a reverse wobble disc and making a forward wobble disc. The process for making a normal, forward wobble CD-R is shown at 188, whereas the process for making a reverse wobble disc is shown at 190. In both cases, a master 192 is made. A father
10 stamper 194 is made from master 192, as appreciated by one of skill in the art. A mother 196 is made from the father stamper 194. The image embossed in the mother 196 is thus a duplicate of the original master 192. To make a forward wobble disc, a son stamper 198 is further made from the mother 196. The son stamper 198 has an image identical to that of the father stamper 194. A forward wobble disc, as shown in FIG. 12, is then made from the son
15 stamper 198. In contrast, a reverse wobble disc 202 is made from the mother stamper.

FIG. 12 further illustrates the difference between manufacturing a forward wobble disc and manufacturing a reverse wobble disc. The son stamper 198' is identical to the son stamper 198 in FIG. 11. The son stamper 198' is used to create a wobble groove on the laser-
20 distal surface 208 of the substrate layer 204, therefore creating a forward wobble disc. Surface 208 may be coated with a dye layer 210 which is further layered with a reflective coating 212. Preferably, the surface 208 is directly coated with the reflective coating 212 without the dye layer 210. The laser beam of the disc reader can travel through the most
25 laser-proximal surface 206 and then the operational layer 204 to reach the surface 208. The surface 208 is the operational surface.

25 In comparison, the reverse wobble discs 214 and 202' are made from the mother stamper. The wobble groove in the reverse wobble disc 214 is positioned at the first or laser-proximal surface of the operational layer 216. Surface 218 is the second or laser-distal surface of the operational layer 216. The cover 222 is capable of focusing the laser beam on the wobble groove. To facilitate the reading of the wobble groove, a reflective layer 220 is
30 deposited on the first surface of layer 216. The first surface of layer 216 is the operational surface. As viewed from the optical pick of the optical disc reader, the operational surface of the reverse wobble disc 214 appears to have the same image as the operational surface 208 of the forward wobble disc.

A reverse wobble disc can be manufactured so that the spiral of the wobble groove moves from the inner diameter of the disc to the outer diameter of the disc when the disc is spun counter-clockwise as viewed from the laser. This configuration is the same as used by a conventional forward wobble disc.

5 In another embodiment, a reverse wobble disc can be prepared using a process similar to process 190 in FIG. 11. However, the wobble groove in the master disc has a reverse image of the wobble groove in the master disc 192. The reverse wobble disc thus prepared has the wobble groove impressed at the laser-proximal surface of its operational layer. A standard CD-R or CD-RW reader can track both the reverse wobble disc of this embodiment and the reverse wobble disc manufactured using the master disc 192. The disc tracking is dependent upon the frequency of the wobble. Likewise, a forward wobble disc can be created using process 188 but with a master disc having a reverse image of the master disc 192.

10 FIG. 13 demonstrates a reverse disc according to one embodiment of the present invention. The reverse disc 240 includes an operational layer 242 which contains a wobble groove 250 at its laser-proximal surface. The wobble groove 250 is coated with a reflective layer 248 which contains reflective material, such as gold. The laser beam 246 can be focused by the cover 244 upon the wobble groove 250 or upon the reflective layer 248. The assembly 240 permits investigational structures to be placed in the analyte section 252 and to be examined by the laser beam 246.

15 For more information about optical discs in which operational structures are positioned in the laser-proximal surface of the operational layer, see U.S. Patent Application Serial No. 09/421,870, entitled "Trackable Optical Discs With Concurrently Readable Analyte Material," which is incorporated herein by reference.

20 In accordance with one aspect of the present invention, various types of data or information can be digitally encoded in the disc assembly. These data or information may be encoded in accordance with industry standards, such as CD or DVD standards, or standards modified therefrom. These encoded data or information may control or facilitate an optical disc reader to track the disc assembly or detect investigational structures that are disposed in the disc assembly.

25 Investigational structures that are disposed in an optical disc assembly can be read or detected using an optical disc reader, such as a CD reader or a DVD reader. As used herein, CD readers include, but are not limited to, CD-ROM readers, CD Recordable (CD-R)

readers, CD-Rewriteable (CD-RW) readers, or any reader capable of reading CD-format discs. Industry standard CD readers may be used in the present invention. Preferably, a standard CD-RW reader or a modification thereof is used in the present invention. As used herein, DVD readers include, but are not limited to, DVD-R readers, DVD-RAM readers, DVD-RW readers, or any reader that can read DVD-format discs. Industry standard DVD readers may be used. As appreciated by one of skill in the art, other CD readers, DVD readers or optical disc readers, including those that have been or will be developed in the future, may be used in the present invention. An optical disc reader may read both CD and DVD discs.

Signals indicative of the presence or other properties of an investigational structure can be generated by an optical disc reader. The disc reader directs a reading beam of electromagnetic radiation, typically a laser beam, to the optical disc assembly in which the investigational structure is disposed. The disc reader can scan the beam over the area in which the investigational structure is held. The scanning beam can be either reflected from or transmitted through the disc assembly. The reflected or transmitted radiation may be acquired by a detector in the disc reader. Radiation thus acquired can be used to produce signals indicative of the presence or other properties of the investigational structure. Different types of lasers with different wavelengths may be used in the present invention. Whereas a standard optical disc reader is used, the disc reader may be connected to circuitry for processing the signals indicative of the presence or other properties of the investigational structure.

Radiation acquired by the detector of the optical disc reader can also be used to generate operational signals, such as focusing servo signals, tracking servo signals, synchronization signals, power control signals, and logic signals. The focusing servo signals can be generated from at least three focusing techniques: critical angle focusing, knife edge focusing and preferably, astigmatic focusing. Tracking servo signals can be generated from at least four types of tracking techniques: one beam-push-pull tracking, three beam outrigger tracking, differential phase detection tracking, and one beam high frequency wobble tracking. Synchronization signals may be generated from at least three different methods: bit clock synchronization or bit pattern synchronization, zoned clocking method, and wobbling groove synchronization. Logic signals can be produced from various optical disc formats. Logic signals can be used to perform position sensing, power control, radial and tangential location, layer sensing, density detection, or other functions.

Preferably, the optical pickup of a standard CD reader is used to both track the optical disc assembly and detect the investigational structure disposed therein. FIG. 14 illustrates an example of the optical pickup of a standard CD reader. The optical pickup 254 contains a laser source 260, which typically is a laser diode. The laser source emits a laser beam 262.

5 The laser beam is collimated by a collimator lens 264. The collimated beam is then directed toward the optical disc through a polarization beam splitter 266. The objective lens 268 focuses the laser beam onto a small spot on a surface in the optical disc. In FIG. 14, the surface is the operational surface of a CD-type optical disc, which contains pits and lands 256. The surface preferably is coated with a reflective layer, so that the laser beam can be reflected therefrom and then directed by the objective lens 268, the mirror 270 and the quarter wave plate 272 to the beam splitter 266. As the light is now polarized in a different direction as compared to the source polarization, it can be directed by the beam splitter towards a photodiode detector 274. A cylindrical lens 276 may serve as an astigmatic element to introduce astigmatism in the reflected laser beam for the purpose of focusing.

10
15 In a preferred embodiment, the laser beam is split into three beams, consisting of a main beam and two tracking beams, and the detector is a quad detector. FIG. 15 shows a quad detector 278, which includes a central quad detector 280 flanked by two additional sensor elements E 281 and F 282. The main beam is centered on a track defined by pits in a CD-ROM disc. The tracking beams fall on either side of the track. By design, the three beams are reflected from the optical disc and then directed to the quad detector 278 such that the main beam falls on the central quad detector 280, which includes sensor elements A, B, C, and D, while the tracking beams fall on the sensor elements E 281 and F 282. The sum of the signals from the central quad detector, i.e. $A + B + C + D$, provides the radio frequency (RF) signal 284, also referred to as the high frequency (HF) or the quad-sum signal. A tracking error signal (TE signal) 288 may be obtained from the difference between E and F. Because astigmatism is introduced by the cylindrical lens astigmatic element, a focus error signal (FE signal) 286 may be obtained from the difference between $A+C$ and $B+D$ signals. Other combinations of the signals from A through F may be obtainable, as appreciated by one of ordinary skill in the art. These combinations of signals may be used to track the disc and read the investigational structure disposed in the disc. A quad detector and a similar optical design may be employed in a DVD reader.

25
30 In one embodiment, the quad-sum signal is used for extracting information indicative of the presence or other properties of the investigational structure disposed in the disc

assembly. Preferably, the disc assembly contains a wobble groove which is trackable by a disc reader. Suitable disc readers include CD-R readers, CD-RW readers or DVD readers. The use of the wobble groove enables the segregation of the tracking signal from the quad sum signal, permitting the quad sum signal to be used to detect signals from investigational structures. If the investigational structure is small enough, an electrical deflection may be detected in the quad-sum signal, while no such a deflection, or a comparatively smaller electrical impulse, will be noted in the tracking signal or the focusing servo signal.

A large investigational feature may also be detected by using the quad-sum signal without losing the tracking of the disc assembly. In such a case, a forward disc, such as that shown in FIGs. 31 and 37, is preferably used. The ability of an operational structure, such as the wobble groove, to permit both disc tracking and analyte detection is herein referred to as segregation of tracking from investigational structures.

The signals produced from a wobble groove can be used by the disc reader to maintain a constant linear scanning velocity at all points on the disc. This allows determination of the dimensional information of the investigational structure that is placed in the disc. Therefore, wobble groove is a preferred operational structure employed in this invention.

Although the above-described embodiment uses the quad-sum signal and the wobble groove, signals other than the quad-sum signal and operational structures other than the wobble groove may be used for the detection of investigational structures. For instance, the focus error signal obtained by the critical angle method, as described in U.S. Patent No. 5,629,514, may be used. The Foucault and astigmatism methods, as described in "The Compact Disc Handbook," by Pohlmann, A-R Editions, Inc. (1992), may also be employed. In addition, the tracking error signals obtained using the single beam push-pull method as described in "The Compact Disc Handbook," by Pohlmann, A-R Editions, Inc. (1992), the differential phase method as described in U.S. Patent No. 5,130,963, or the single beam high frequency wobble method can be used for the present invention. The block error rate information, such as those used by a CD reader to reduce the effect of scratches on a CD surface, or the movement of the focusing servo may be used.

For more optical pickup designs and operational signals that may be used in the present invention, see "Compact Disc Technology," by Nakajima and Ogawa, IOS Press, Inc. (1992); "The Compact Disc Handbook," by Pohlmann, A-R Editions, Inc. (1992); "Digital Audio and Compact Disc Technology," by Baert et al. (eds.), Books Britain (1995);

“CD-Rom Professional’s CD-Recordable Handbook: The Complete Guide to Practical Desktop CD,” Starrett et al. (eds.), ISBN:0910965188 (1996). All these references are incorporated herein in their entirety by reference.

U.S. Provisional Application Serial Nos. 60/270,095 and 60/292,108 further detail how to extract or process signals indicative of the presence of an investigational structure disposed in an optical disc assembly. Both applications are incorporated herein by reference.

FIG. 16 and FIG. 17 illustrate a measurement of investigational structures using an optical disc assembly according to one embodiment of the present invention. The analyte section is laser-proximal to the operational layer, and is positioned between the operational surface and a lens layer. FIG. 16 depicts the position of the investigational structures **800** with respect to the tracks **802**, **804**, **806** and **808**. The investigational structures **800** are held in the analyte section and positioned on the operational surface. The investigational structures **800** are 2.8 micrometer magnetic beads. Tracks **802**, **804**, **806** and **808** are embossed in the operational surface. Each track preferably is in the form of a wobble groove.

FIG. 17 shows the HF signals **802'**, **804'**, **806'** and **808'** that are acquired along the tracks **802**, **804**, **806** and **808**, respectively. The HF signals shown in FIG. 17 have been digitalized and buffered. The HF signals **802'**, **804'**, **806'** and **808'** demonstrate the existence as well as the approximate dimension of the investigational structures **800**.

FIGs. 18 through 24 illustrate the measurement of a gnat wing disposed in an optical disc assembly. FIG. 18 is a cross-sectional view taken perpendicular to a radius of the optical disc assembly. In FIG. 18, the optical disc assembly includes an operational layer **814**, a first reflective layer **816**, a second reflective layer **810** and a cover **812**. The analyte section **826** is located between the second reflective layer **810** and the operational layer **814**. The operational surface **818** is the laser-distal surface of the operational layer **814**. The operational surface **818** is embossed with tracks of operational structures, preferably, wobble grooves. The operational surface **818** is covered by the reflective layer **816**. The operational surface has a cut-away area **824** which may lack the reflective coating **816**. The gnat wing **822** is located in the analyte section **826** and positioned upon the cut-away area **824**. The laser beam **820** can pass through the operational layer **814**, the cut-away area **824** and the gnat wing **822**, and then be reflected by the reflective layer **810**.

FIG. 19 diagrammatically shows the position of the gnat wing **822** relative to the tracks embossed in the operational surface. **T1** denotes a track upon which the gnat wing is

positioned. FIG. 20 shows the HF signal acquired along the track **T1**. The HF signal has been digitalized and buffered.

In FIG. 19, the Y-axis, labeled with "TRACKS," represents the number of tracks along a radius of the disc assembly. The X-axis, labeled with "TIME (SAMPLES)," represents the number of sampling along a track. For instance, the gnat wing can be sampled by the disc reader along the track **T1** at least about 800 times (from sample number 1550 to sample number 2350).

FIG. 21 shows the position of the gnat wing **822** relative to the tracks **T2** and **T3**. FIG. 22 demonstrates the HF signals for a series of consecutive tracks between **T2** and **T3**.

FIG. 24 is a high density compilation of the HF signals for the tracks across the gnat wing. The image of the gnat wing appears in FIG. 24. FIG. 23 shows the position of the gnat wing **822** relative to the tracks.

In accordance with another aspect of the present invention, the optical disc reader includes an objective lens focusing mechanism which is capable of focusing the disc reader's laser onto different surfaces in the disc assembly. In one embodiment, the focusing servo **290** shown in FIG. 25 includes a coil and magnet casing surrounding the objective lens. The objective lens **292** directs the laser **294** onto the surface **296** of the disc. Then the laser light travels through a lens layer, and focuses onto the operational surface **298**. The operational surface contains operational structures, such as wobble grooves, or pits and lands. The objective lens is held by a brace **302** which is part of the moving coil controlled by the magnet **306**. The magnet **306** is activated by the signal generated when the laser light **294** returns from the disc to the detector of the disc reader. The moving coil **304** and the objective lens can move within the range **308**. The range **308** controls the range of the laser's focal zone.

The focusing servo of the present invention may move the laser's focal point across the laser's focal zone. The laser's focal point may represent the point where the focusing servo finds a maximal amount of light returned to the disc reader's detector. The location of the laser's focal point therefore may be affected by the reflective properties and other optical properties of various elements contained in the optical disc assembly. For instance, where the optical disc assembly is based on the modification of a conventional CD-R, the focal point may be at the reflective layer which contains gold or other reflective material.

More than one reflective layer, including semi-reflective layers, may be constructed within the laser's focal zone. The reflective layers in the focal zone can create a level of

signals, the voltage of which is affected by the position of the focal point and the reflectivity of the reflective layers. The focusing servo may be able to search for a focal point from which the light returned to the detector is maximal.

In a preferred embodiment, the operational structures in the optical disc assembly are designed to provide an improved detection for the investigational structures. The investigational structures may be cells, microorganisms or any other biological, biochemical, or chemical specimens. In one instance, the pits, lands, or wobble grooves are reconfigured to enhance tangential resolution. In another case, the pits are shortened or the wobble is changed to provide a lower scanning speed in order to increase the radial resolution. In yet another instance, the lens layer is changed to PMMA or other material to improve optical response. The lens layer may also be made thicker or thinner to make the focal spot smaller or larger, therefore providing higher or lower energy distributions on the detector. The operational structures may be interleaved to provide dynamic responses for enhancing imaging.

The present invention contemplates a variety of embodiments of the optical disc assembly. For instance, the optical disc can be a forward disc or a reverse disc. The optical disc can have more than two reflective layers. The operational structures can include pits, lands, grooves, wobble grooves, dye marks, chevron marks, or any combination thereof. The operational structures may act as phase components or create interference patterns that provide tracking and synchronization information to the disc drive. The operational structures can be in a CD format (including a CD-R and CD-RW format), a DVD format (including a DVD-R format, a DVD-RW format and a DVD-RAM format), any combination thereof, or any other optical disc format. The operational structures can be physically imprinted in a surface of the operational layer, or encoded in a hologram. A custom format for operational structures may also be used, and the disc assembly is read by a custom decoding device. Different surfaces in the optical disc assembly can be metalized or coated with materials with a variety of reflective properties. The coatings may be reflective, semi-reflective, transmissive, semi-transmissive, or anti-reflective. The materials used in the various layers may be dielectric or non-dielectric. Moreover, the operational layer may be created using different processes, such as molding, electroforming, or web manufacturing.

The analyte section of the optical disc assembly can be configured to receive an insert which holds the analyte of interest. The insert can be glass or plastic. The insert may be a sample slide regularly used for examining biological, biochemical, or chemical samples. The

insert may be replaceable or integrated with the disc assembly. The insert may hold chemical, biological or other physical specimens. Chemical or biological reactions, including molecule-molecule bindings or enzymatic reactions, can be performed either on the insert or in the analyte section. Products of these chemical or biological reactions may generate optical effects on the incident laser light which can in turn be detected by the disc reader. The insert may function as a cover layer or a lens layer.

The analyte section may include investigational structures which are replaceable or integrated with the disc assembly. The investigational structures may contain light absorbing, light reflecting materials, or anti-reflective materials, so that they may be detectable by the optical pickup of the disc reader. The focusing servo may search for the focal point with maximal return light. Such a focal point may depend on the reflectivity of the investigational structures.

The analyte section or any other layer in the disc assembly may contain channels or chambers, including microfluidic channels, through which analytes, investigational features or reactive components may enter or exit the analyte section. These channels or chambers may also be used to hold the analytes or investigational features for investigation.

The optical disc assembly of the present invention preferably includes a lens layer. The thickness of the lens layer may be about 1.1 mm to 1.3 mm, including about 1.2 mm. Such a lens layer may be used in a disc assembly that is modified from a CD-type disc. The thickness of the lens layer may also be about 0.6 mm, and can be used in a disc assembly modified from a DVD-type disc. The refractive index of the lens layer preferably is selected so that the laser of the disc reader can be focused in a desired manner. The lens layer may be made from a variety of materials, including glass, plastic, PMMA, polystyrene, polycarbonate, or dyed material. The lens layer may be flat or non-flat. The lens layer may be homogeneous or non-homogenous. The lens layer may be incorporated into the operational layer. The lens layer may contain at least part of the analyte section.

The lens layer may also contain molded features, such as cavities, channels or waveguides. The lens layer may function as a cover to protect the operational layer or other layers of the disc assembly. The lens layer may provide physical support for other layers in the disc or for the investigational structures or the analytes that are associated with the disc. The lens layer may be either laser-proximal or laser-distal to a reflective layer or a semi-reflective layer.

In a preferred embodiment, the operational layer includes or consists of a hologram. The operational structures are encoded in the hologram. Preferably, the hologram is a reflective hologram, and is protected by a transparent protective coating located laser-proximal to the hologram. FIG. 26 shows an optical disc assembly containing a reflective
5 hologram 512 which is protected by a transparent protective coating 514. The hologram encodes the operational structures, such as wobble grooves, that are required by the operation of the optical disc reader. When a laser beam 516 is reflected from the hologram physical plane 512, it appears as though the encoded operational structures, such as wobble grooves in a correct orientation, are present at the hologram image plane 518. The hologram image
10 plane 518 can be located substantially confocal with the investigational structure 520. The investigational structure 520 may be positioned on the laser-distal surface 522 of the lens layer 524.

The laser beam may be focused on the image plane 518 which is shared by both the investigational structure 520 and the encoded operational structures. Therefore, light from
15 the image plane 518 may enable the disc reader to generate both the operational signals, such as the signals used for disc tracking, and the investigational signals that are indicative of the presence of the investigational structure. This feature allows the optical disc reader to track the disc and detect the investigational structure concurrently and discriminably. The larger the illuminating laser spot 526 on the hologram, the better the image of the operational
20 structures as appeared in the image plane 518. Therefore, the laser preferably is not tightly focused on the hologram physical plane. Typically, a portion of the hologram physical surface may be sufficient to generate the entirety of the image of the operational structures that are interferometrically encoded in the hologram.

The hologram image plane may be positioned not concurrently with the
25 investigational structure, provided that the operational structures, such as the wobble groove, can be concurrently detectable with the investigational structure. The image plane of the hologram may be either laser-proximal or laser-distal to the investigational structure. In addition, the hologram image plane may be either laser-proximal or laser-distal to the hologram's physical plane.

30 Preferably, the hologram is replaceable or reversibly attachable to the disc assembly. This permits the hologram to be mass-produced using a high-speed holographic printing process. This also permits the re-use of the hologram or other parts of the disc assembly.

In another preferred embodiment, the operational structures in the optical disc assembly are configured and organized in accordance with the “zoned constant linear velocity” (ZCLV) format. The ZCLV format is detailed in various industry standards, including the DVD-RAM specification. FIG. 27 schematically illustrates the ZCLV format in a circular disc. The ZCLV disc in FIG. 27 is divided into multiple zones 490 across the range 492. Although only five zones are shown in FIG. 27, actual ZCLV format discs may have different numbers of zones. For instance, the DVD-RAM ZCLV format allows 24 zones.

Each of the zones 490 is divided into multiple sectors 494. Inner zones have fewer sectors than outer zones, because the radii of inner zones are less than the radii of outer zones. The optical disc reader can scan each zone at a constant rate. In addition, the optical disc reader can rotate the ZCLV disc faster when it scans the inner zones than when it scans the outer zone. Therefore, the optical disc reader may maintain a substantially constant scanning rate for all the zones in a ZCLV disc.

FIG. 28 shows an enlarged perspective view of a portion of one of the sectors of the ZCLV disc. The operational structures consist of multiple tracks 498 which are arranged radially within the sector. Each track has header information embossed in a “pre-groove” area 500. The pre-groove area is followed by a “wobbled land and groove” area 504 which contains the wobble groove 498 and the wobbled land 502. Operational structures in a ZCLV format may be holographically encoded in a hologram.

FIG. 29 shows a ZCLV-formatted optical disc assembly associated with analytes or investigational structures 510. Analytes or investigational structures 510 may be deposited within the “wobbled land and groove” area 508. The embossed header information in the pre-groove area 506 can be used to store information for identifying or controlling a desired measurement of the analytes or investigational structures 510. Accordingly, different sectors in the same ZCLV disc may be used to perform different measurements or assays.

In one embodiment, the zones in a ZCLV formatted disc can be mastered in such a way as to provide either a highly reflective surface, a partly reflective surface, or a non-reflective surface. In each case, the pre-header information can provide tracking and location information. The pre-header information may also provide identification information for the nature of the zone. In addition, the pre-header information may encode information relating to the software or firmware that is applied to the zone. The characteristics of the

investigational structures (such as their reflectivity, absorptivity, or transmissivity) can be determined by the disc laser as it scans over each zone.

In a preferred embodiment, the optical disc assembly includes a semi-reflective layer which is both transmissive and reflective to the laser light. The semi-reflective layer is usually a thin layer of reflective or semi-reflective material, such as silicon, tellurium, selenium, bismuth, aluminum, silver, copper or other suitable metals or alloys. The thickness of the layer may range from 10 nm to 100 nm. The reflectivity of the semi-reflective layer may range from about 18% to 30%. The reflectivity may also range from about 30% to 40%. The semi-reflective layer may be used to coat operational structures. Whereas the reflectivity of the semi-reflective layer is low, for instance, below about 30%, a CD-RW reader or a DVD reader preferably is used to read the operational structures that are coated with the semi-reflective layer.

FIG. 30 illustrates a forward optical disc assembly which includes a semi-transmissive, semi-reflective layer. The semi-transmissive, semi-reflective layer 530 is placed on the operational structures which are embossed in the laser-distal surface of the operational layer 528. Reference numeral 534 shows an operational structure coated with the layer 530. The reflectivity of the layer 530 can return sufficient light to render the operational structures detectable by the disc reader. The laser beam 532 can also pass through the layer 530 to enter the analyte section 531 which is configured to hold the analyte 538. The analyte section 533 and the analyte 538 are within the laser's focal zone 536. The laser beam 532 can be reflected back from another reflective layer 533 which is laser-distal to the semi-transmissive, semi-reflective layer 530. This latter reflected light may carry signals indicative of the presence of the analyte. The laser's focus is able to roam within the focal zone 536.

FIG. 31 illustrates a forward transmissive pass-through disc assembly which permits the laser light 540 to pass through the top refractive layer 542 to a top detector. The laser 540 is focused by the lens layer 544, which is also the operational layer. The operational features are coated with a partly reflective and partly transmissive layer 548. Reference numeral 546 shows a coated operational feature. The analyte 550 is placed in the analyte section 552 which is situated between the lens layer 544 and the top refractive layer 542. Samples including solutions may be directed to and expelled from the analyte section 552 through channels or other means. The laser's focus, driven by the laser's focusing servo, can roam

within the focal zone 554. The coated operational features and the analyte 550 are both placed within the focal zone and can be detected concurrently and discriminably.

In accordance with another aspect of the present invention, the optical disc assembly may contain channels or chambers capable of transporting analytes, reactive components or mediums to and from the analyte section. Analytes and other components can be mixed within these channels or chambers. Preferably, the analyte section also contains at least one chamber or channel which is capable of holding the analytes for investigation. More preferably, the operational surface contains a cut-away area which may be either laser-proximal or laser-distal to the analyte section and which may be adjacent to the analyte section. Most preferably, the analyte section includes a cut-away area as one surface.

FIG. 32 demonstrates a reverse disc assembly 578 including fluidic channels. The lens layer 570 is laser-proximal to the operational layer 572. The operational structures are embossed in the laser-proximal surface of the operational layer 572. The plane 574 depicts the plane upon which the operational surface and the operational structures are located. The operational layer 572 contains channels 576 through which analytes or investigational structures can enter or exit the analyte section. The analyte section includes the channel 582 which can be located either laser-proximal or laser-distal to the operational surface. The arrows associated with 576 and 582 indicate the direction of fluidic flow in the channels. The channel 582 and the operational surface are located within the focal zone 584.

FIG. 33 shows another reverse optical disc assembly 580 in which the analyte section, which includes channel 588, is either laser-proximal or laser-distal to a cut-away area. The operational surface is positioned on the plane 574'. The cut-away area is in the operational surface which is the laser-proximal surface of the operational layer 572'. The cut-away area preferably lacks operational structures or reflective coatings. Channels 576' direct the fluidic flow to and from channel 588. The operational surface, the cut-away area and channel 588 are within the focal zone 584'. 570' denotes the lens layer.

FIG. 34 depicts a preferred reverse optical disc assembly. The lens layer 590 focuses the laser beam 592 either on the reflective layer 594 or on the analyte 596. The reflective layer 594 coats the operational structures located at the laser-proximal surface of the operational layer 604. The analyte 596 resides in the analyte section which consists of the chamber 598. The chamber 598 is laser-proximal to the operational structures and laser-distal to the lens layer. The adhesive layer 600 binds the operational layer 604 to the lens layer 590. The channels 602 are etched into the operational layer 604. Analytes can enter

and leave the analyte section 598 through channels 602. The operational layer 604 is covered by the cover 606. The operational structures and the analyte section are with the laser's focal zone 608.

FIG. 35 demonstrates a forward optical disc assembly 618 which includes fluidic channels 616 and 620. The operational layer 610 serves as a lens layer and is capable of focusing the laser beam 614 on the operational structures 612. The operational structures 612 are located at the laser-distal surface of the operational layer 610. Channels 616, contained in the cover, can introduce analytes into the analyte section which includes the channel 620. The channel 620 can be either laser-proximal or laser-distal to the operational structures. Both the operational structures and the channel 620 are within the focal zone 622.

FIG. 36 shows a forward disc assembly 621 including a cut-away area 628 in the operational surface. The cut away area 628 is in the laser-distal surface of the operational layer 610', and preferably lacks operational structures or reflective coatings. Other areas in the laser-distal surface of the operational layer 610' are contained with the operational structures 612'. The channel 626 in the analyte section can be either laser-proximal or laser-distal to the cut-away area 628. The focal zone 622' encompasses the operational structures 612', the cut-away area 628, and the channel 626.

FIG. 37 illustrates a preferred embodiment of a forward optical disc assembly which includes analyte channels 666. The analyte channels 666 are in or immediately above the lens layer 658. These channels are connected to the analyte section 668. The lens layer 658 is attached to the operational layer 662 through the adhesive layer 660. The lens layer 658 is laser-proximal to the operational layer 662. The operational structures at the laser-distal surface of the operational layer 662 are coated with a semi-reflective, semi-transmissive layer 664. The analyte section 668 is configured to receive analytes 670, and is located laser-distal to the semi-transmissive layer 664 and laser-proximal to another reflective layer 678. The reflective layer 678, which preferably is fully reflective, is laser-proximal to the cover 680. Laser beam 676 can pass through the lens layer 658, the adhesive layer 660, the operational layer 662 and the semi-transmissive layer 664 to focus on the analytes 670 or the reflective layer 678. The laser 676 can be reflected from the reflective layer 678. The laser focal point can move across the focal zone 674. Light reflected from layer 664 may carry operational information, whereas light reflected from layer 678 may carry investigational information.

FIG. 38 schematically demonstrates another embodiment of a forward optical disc assembly. The analyte section includes an analyte channel which can be located either laser-

proximal or laser-distal to the cut-away area 698. The cut-away area 698 is at the laser-distal surface of the operational layer 692. The cut-away area preferably lacks operational structures or reflective coatings. Other areas in the laser-distal surface of the operational layer 692 include the operational structures 694. In FIG. 38, the cut-away area 698 is laser-proximal to the plane upon which the operational structures reside. Channels 696 are created in the cover layer of the disc assembly. Analytes or reaction components can enter or exit the analyte channel through channels 696. The laser's focal zone 693 encompasses the operational structures 694, the cut-away area 698, and the analyte channel. One of skill in the art will appreciate that, in view of FIG. 38, the cut-away area 698 may also be located laser-distal to the plane upon which the operational structures are disposed.

FIG. 39 illustrates a preferred embodiment of a forward optical disc assembly including a cut-away area. The cut-away area is laser-proximal to the plane upon which the operational structures reside. The operational structures are positioned at the laser-distal surface of the operational layer 724, and are coated by a reflective or semi-reflective layer 726. The cut-away area preferably lacks operational structures or the coating 726. The analyte section includes a chamber 728 which is etched into the operational layer 724. The cut-away area constitutes the most laser-proximal surface of the chamber 728. Fluidic flow 730 enters into the chamber 728 through channels 732 which cut through the cover layer 734. A reflective layer 736 is located laser-proximal to the layer 734 but laser-distal to the operational structures. The laser 738 encounters and detects the first analytes 740 which are deposited on the cut-away area. The fluidic flow 730 may also mix the first analytes 740 with the second analyte 742. The interaction between the analytes 740 and the analyte 742 may generate detectable signals. The laser's focal zone 744 encompasses the chamber 728. As appreciated by one of skill in the art, channels 732 may be created with the operational layer 724.

In one embodiment, the disc is a hybrid disc in that the operational surface includes at least two different formats of operational structures. For instance, the operational surface may include operational structures in a CD format as well as operational structures in a DVD format. The disc reader may read both formats. Data facilitating or regulating the disc reader to read different formats of operational structures may be encoded or embossed in the disc.

In a preferred embodiment, data other than operational structures can be encoded or embossed in the operational surface or other surfaces in the disc assembly. These data may provide control information for the disc reader to read or detect the investigational structures.

These data may regulate the measurement of investigational structures, for instance, by controlling valves which can manage fluidic flows in a fluidic circuit. These data, as well as the operational structures or other information, may be written to the disc before or after the investigational structures are read or detected.

5 The disc assembly may be used for detecting biological suspensions such as blood, urine, saliva, amniotic fluid, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid. Environmental and chemical samples may also be assayed using the disc assembly. The disc assembly may include embossed features, placed features or etched features.

10 The disc assembly may be a null type disc, a modified disc based on industry standard disc, such as a modified CD-R or a modified CD, or a disc based on a custom format. Preferably, the disc assembly does not have the dye layer as used in a CD-R or a CD-RW disc.

15 In one embodiment, data used to identify the type of the disc assembly can be encoded in the disc assembly. For instance, a disc identification number (DIN) may be digitally encoded in the disc to describe the type of the operational layer, such as a forward wobble disc or a reverse wobble disc. The DIN may also be used to describe the design of the analyte section, the adhesive layer or the cover of the disc.

20 In another embodiment, the disc assembly includes an operational layer, an adhesive layer and a cover. The operational layer, the adhesive layer, or the cover of the disc may contain channels, cavities, slots, openings, or holes. The thickness of the adhesive layer may be between 25 and 500 micrometers, including within the range of about 25 micrometers to 100 micrometers, such as 50 micrometers. The adhesive material may be pressure sensitive, and can be made from acrylic or silicone. The cover layer may be coated with reflective
25 materials such as gold.

An analyte chamber may be located within the adhesive layer. The operational layer or the cover may contain channels that are connected to the analyte chamber. Analytes, components reactive thereto or mediums can be introduced through these channels to the analyte chamber.

30 FIG. 40 illustrates an exploded perspective view showing the principle layers of a forward disc assembly according to one embodiment the present invention. The operational layer 966 is laser-proximal to the adhesive layer 958 which is laser-proximal to the cover 950. The operational surface 968 is coated with a semi-reflective, semi-transmissive layer.

The operational layer 966, the adhesive layer 958 and the cover 950 includes a central hole 964, 962 and 954, respectively. The disc assembly can be coupled to a disc reader through these holes, for example, by clamping to a motor shaft. The adhesive layer includes an opening 960, which forms an analyte chamber when these layers are assembled together. The adhesive layer has a depth of about 100 micrometers. Investigational structures can be manipulated or retained in the analyte chamber formed by the opening 960. The cover 950 includes the ports 952 and 956. When the disc is assembled, these channels are connected to the analyte chamber 960. Investigational structures or other reaction components may enter or exit from the chamber 960 through the ports 952 and 956. Both the operational layer and the cover layer may be made from polycarbonate. The laser beam may pass through the disc assembly and be read by a top detector. Alternatively, a reflective layer may be coated at the laser-proximal surface of the cover 950 so that the laser beam can be reflected back therefrom.

As appreciated by one of skill in the art, a reverse wobble disc assembly can be similarly constructed as shown in FIG. 40. In addition, channels or openings, through which investigational structures or reaction components can be introduced into the analyte section, may be created in the operational layer. Operational structures may be created in the laser-proximal surface of the cover layer 950.

Channels, cavities, inlet and vent ports, or other structures in the operational layer, the adhesive layer or the cover may be in a variety of configurations. FIG. 41 shows an operational layer 902 which has four slots 900. FIG. 42 shows an adhesive layer 904 which has a series of openings 906. These openings can form analyte chambers in the assembled disc. These different chambers may be connected to different channels located in either the cover layer or the operational layer. The assembled disc may perform different assays in different analyte chambers.

Data encoding design information that identifies the type of the disc assembly may be stored in the disc assembly and readable by the disc reader. Such information may also be printed on a surface of the disc assembly. For instance, a disc identification number (DIN) can be used to identify the type of the disc assembly. The number may include alphanumeric characters that are subdivided into different fields. One field may describe the main specifications of the operational layer. The main specifications include whether the disc is a reverse disc or a forward disc, and the particular disc design type for the operational layer. The main specifications may also include an identification number for the master of the

operational layer. A second field of the DIN may represent various modifications or other variables of the operational layer. The variables include the placement of the cut-away areas and the number of analyte chambers. In addition, the second field of the DIN may describe the position of the adhesive layer or techniques or materials used in making the disc

5 assembly. The designs of the cover layer may also be described in the second field. A third field may include , which can be used to identify each disc. This serial number may be in the order of manufacture of the disc.

It should be understood that the above-described embodiments are given by way of illustration, not limitation. Various changes and modifications within the spirit and scope of
10 the present invention will become apparent to those skilled in the art from the above description. In addition, the reader's attention is directed to the provisional applications from which the present application claims priority. The contents of all these provisional applications are incorporated herein by reference.

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201